

*A*

Patent  
Attorney's Docket No. 012712-652

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

REQUEST FOR FILING CONTINUATION/DIVISIONAL  
APPLICATION UNDER 37 C.F.R. § 1.53(b)

Box PATENT APPLICATION  
Assistant Commissioner for Patents  
Washington, D.C. 20231

Sir:

This is a request for filing a ☐ continuation ☒ divisional application under 37 C.F.R.  
§ 1.53(b) of pending Application No. 09/080,349 filed on May 18, 1998, for TREATMENT  
OF T CELL MEDIATED AUTOIMMUNE DISORDERS, by the following named inventor(s):

- (a) Full Name Randolph J. Noelle
- (b) Full Name Eric Claassen
- (c) Full Name \_\_\_\_\_

- ☐ The entire disclosure of the prior application from which a copy of the oath or declaration is supplied herewith is considered as being part of the disclosure of the accompanying application and is hereby incorporated by reference therein.
- ☒ This application is being filed by less than all the inventors named in the prior application. In accordance with 37 C.F.R. 1.63(d)(2), the Commissioner is requested to delete the name(s) of the following person or persons who are not inventors of the invention being claimed in this application.

- (a) Full Name Eric Claassen
- (b) Full Name \_\_\_\_\_
- (c) Full Name \_\_\_\_\_

1. ☒ Enclosed is a copy of the prior Application No. 09/080,349 as originally filed on May 18, 1998, including copies of the specification, claims, and drawings as filed.
2. ☐ Enclosed is a revised prior application and a copy of the prior executed oath or declaration as filed. No new matter has been added to the revised application.
3. ☒ One statement(s) claiming small entity status ☒ are enclosed ☒ were filed in prior Application No. 08/481,735, filed on June 7, 1995 (parent of 09/808,349).

4. ☒ The filing fee is calculated below ☒ and in accordance with the enclosed preliminary amendment:

| CLAIMS   |                  |            |                 |             |                 |
|--|------------------|------------|-----------------|-------------|-----------------|
|  | NO. OF<br>CLAIMS |            | EXTRA<br>CLAIMS | RATE        | FEE             |
| Basic Application Fee  |                  |            |                 |             | \$760.00        |
| Total Claims   | 10               | MINUS 20 = | 0               | x \$18.00 = | 0.00            |
| Independent<br>Claims  | 2                | MINUS 3 =  | 0               | x \$7800 =  | 0.00            |
| If multiple dependent claims are presented, add \$270.00                   |                  |            |                 |             | 0.00            |
| Total Application Fee  |                  |            |                 |             | 380.00          |
| If small entity status is claimed, subtract 50% of Total Application Fee   |                  |            |                 |             | 0.00            |
| Add Assignment Recording Fee of \$40.00 if Assignment document is enclosed |                  |            |                 |             | 0.00            |
| <b>TOTAL APPLICATION FEE DUE</b>   |                  |            |                 |             | <b>\$380.00</b> |

5. ☐ Charge \$ \_\_\_\_\_ to Deposit Account No. 02-4800 for the fee due.
6. ☒ A check in the amount of \$ 380.00 is enclosed for the fee due.
7. ☒ The Commissioner is hereby authorized to charge any appropriate fees under 37 C.F.R. §§ 1.16, 1.17 and 1.21 that may be required by this paper, and to credit any overpayment, to Deposit Account No. 02-4800. This paper is submitted in triplicate.
8. ☐ Cancel in this application original claims \_ of the prior application before calculating the filing fee. (At least one original independent claim must be retained for filing purposes.)
9. ☒ Amend the specification by inserting before the first line the sentence: --This application is a ☐ continuation, ☒ divisional, of Application No. 09/080,34, filed May 18, 1998, which is a continuation of 08/481,735, filed June 7, 1995, the contents of which are incorporated herein by reference.--
10. ☐ Transfer the drawings from the pending prior application to this application and abandon said prior application as of the filing date accorded this application. A duplicate of this paper is enclosed for filing in the prior application file. (May only be used if signed by person authorized under 37 C.F.R. § 1.138 and before payment of issue fee.)
11. ☒ New **formal** drawings are enclosed.

12. ☐ The prior application is assigned of record to Trustees of Dartmouth College.
13. ☐ Priority of Application No.      filed on      in      (country) is claimed under 35 U.S.C. § 119.
- ☐ The certified copy of the priority application
- ☐ is enclosed
- ☐ was filed on      in prior Application No.     , filed on
- ☐ has not yet been filed.
14. ☒ A preliminary amendment is enclosed.
15. ☒ Also enclosed Information Sheet
16. ☒ The power of attorney in the prior application is to E. Joseph Gess.
- a. ☒ The power appears in the original papers in the prior application.
- b. ☐ Since the power does not appear in the original papers, a copy of the power in the prior application is enclosed.
- c. ☒ Recognize as Associate Attorney Robin L. Teskin, Registration No. 35,030.
- d. ☒ Address all future communications to: (May only be completed by applicant, or attorney or agent of record.)

E. Joseph Gess  
BURNS, DOANE, SWECKER & MATHIS, L.L.P.  
P.O. Box 1404  
Alexandria, Virginia 22313-1404

December 31, 1998

Date

By: Robin L. Teskin  
Robin L. Teskin  
Registration No. 35,030

ADDRESS OF  
SIGNATOR:

BURNS, DOANE, SWECKER & MATHIS, L.L.P.  
P.O. Box 1404  
Alexandria, Virginia 22313-1404  
(703) 836-6620

☐ inventor(s)  
☐ assignee of complete interest  
☐ attorney or agent of record  
☒ filed under 37 C.F.R. § 1.34(a)

**IN THE UNITED STATES PATENT AND TRADEMARK OFFICE**

In re Patent Application of )  
)  
Randolph J. NOELLE ) Group Art Unit: Unassigned  
)  
Application No. Unassigned ) Examiner: Unassigned  
)  
Filed: December 31, 1998 )  
)  
For: TREATMENT OF T CELL )  
MEDIATED AUTOIMMUNE )  
DISORDERS )

**PRELIMINARY AMENDMENT**

Assistant Commissioner for Patents  
Washington, D.C. 20231

Sir:

Prior to examination, please first amend the above-identified application as follows:

**IN THE CLAIMS**

In Claim 2, line 2, please delete "multiple sclerosis, EAE".

Kindly cancel Claim 3.

**REMARKS**

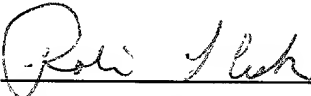
The above amendment and cancellation of Claim 3 are respectfully requested in order to better emphasize the preferred embodiment of the invention.

Application No. Unassigned  
Attorney's Docket No. 012712-652

If any questions arise in connection with this application, kindly contact the undersigned attorney at the telephone number listed below.

Respectfully submitted,

BURNS, DOANE, SWECKER & MATHIS, L.L.P.

By:   
Robin L. Teskin  
Registration No. 35,030

P.O. Box 1404  
Alexandria, Virginia 22313-1404  
(703) 836-6620

Date: December 31, 1998

## **TREATMENT OF T CELL MEDIATED AUTOIMMUNE DISORDERS**

### **Government Funding**

The work leading to this invention was supported by one or more grants from the U.S. government. The government may have rights in the invention.

### **Background of the Invention**

Autoimmune diseases are characterized by attack of the immune system of an individual against its own tissues. Autoimmune diseases usually result from breakdown of tolerance of the immune system to its own antigens. The specific antigens recognized by the immune system in the various autoimmune diseases can be present systematically or they can be organ specific. For example, systemic lupus erthematosus (SLE) is characterized by the presence of autoantibodies to DNA, ribonucleoproteins, histones, and other molecules that are not organ specific. Other autoimmune diseases are characterized by the destruction of mostly one organ. Such autoimmune diseases include type I diabetes, in which the insulin producing  $\beta$  cells of the islets of Langerhans in the pancreas are destroyed.

In some autoimmune diseases, tissue destruction occurs primarily as a result of the production of high levels of autoantibodies. Such diseases include rheumatoid arthritis, characterized by destruction of the joint cartilage and inflammation of the synovium. Patients with rheumatoid arthritis have an accumulation of immune complexes in their joints which are formed by association of autoantibodies against the Fc portion of IgG and IgG molecules. These immune complexes activate the complement cascade which results in tissue damage. Myasthenia gravis, a disease of progressive muscle weakness, is caused by the production of autoantibodies reactive to acetylcholine receptors in the motor end plates of neuromuscular junctions.

In other autoimmune diseases, tissue destruction does not appear to be primarily mediated by production of autoantibodies, but rather by auto-reactive T lymphocytes. For example, experimental allergic encephalomyelitis (EAE), an animal model for multiple sclerosis, and characterized by demyelination in the brain and the spinal cord, can be induced in naive animals by transfer of CD4+ T cells from diseased animals. Thus, it is generally considered that EAE represents a T cell mediated autoimmune disease, rather than a B cell mediated autoimmune disease (Ben-Nun, A. et al. (1981) *Eur. J. Immunol.* 11, 195).

Multiple sclerosis (MS) is a common demyelinating disease of the brain and spinal cord. It is a progressive disease that is characterized by remissions and exacerbations of neurologic dysfunction affecting different regions of the central nervous system. The symptoms of the disease result from a focus of inflammatory demyelination, which later

forms a scar, appearing as a "plaque" in the white matter of the brain, brain stem or spinal cord. Presently, there is no definitive diagnostic test available for MS and diagnoses and treatment regimes are being formulated based on such factors as the extent of a patient's symptoms and/ or the age of the patient at the time of onset of the exacerbations of neurologic dysfunction.

Patients having MS typically have been treated with steroids with a goal of either sending the patient into remission or slowing the progression of the disease in the patient. Other drugs have been used to treat particular symptoms of the disease, e.g. muscle relaxants. Recent developments in treatments available for MS include the administration of beta-interferon. Beta interferon has shown some promise for slowing the progression of the disease. However, effective treatments for MS are still needed.

### **Summary of the Invention**

This invention pertains to methods for treating (therapeutically or prophylactically) a T cell mediated autoimmune disorder, such as multiple sclerosis. The method comprises administering to the subject a therapeutically or prophylactically effective amount of an antagonist of a receptor on a surface of a T cell which mediates contact dependent helper effector functions. In a preferred embodiment the antagonist administered is an antibody or fragment thereof which specifically binds to the T cell receptor gp39.

### **Brief Description of the Figures**

*Figure 1* is a graphical representation of DAS units measured daily in mice injected at day 0 with 75  $\mu$ g (Panel A) or 300  $\mu$ g (Panel B) of PLP-peptide with an anti-gp39 antibody (black bars) or with PBS (grey bars) showing that anti-gp39 administration prevents the development of experimental allergic encephalomyelitis EAE.

*Figure 2* is a graphical representation of the percentage suppression of EAE induction in mice injected with PLP-peptide at day 0 and further injected with anti-gp39 antibodies (black bars) or PBS (grey bars) at days 0, 2, and 6, or at days 4, 6, and 8, or at days 7, 9, and 11, showing that administration of anti-gp39 after induction of the disease significantly prevents EAE.

*Figure 3* is a graphical representation of DAS units measured daily in mice transplanted with donor spleen cells from mice injected with PLP-peptide and anti-gp39 antibodies (black bars) or with donor cells from mice injected with PLP-peptide alone (grey bars) and injected with PLP-peptide.

### **Detailed Description of the Invention**

This invention pertains to the treatment of T cell mediated autoimmune disorder such as multiple sclerosis. The disease is treated by administering an antagonist to a receptor on the surface of T cells which mediates contact dependent T cell helper effector function.

As defined herein, a "molecule or receptor which mediates contact dependent helper effector functions" is one which is expressed on a Th cell and interacts with a ligand on an effector cell (e.g., a B cell), wherein the interaction of the receptor with its ligand is necessary for generation of an effector cell response (e.g., B cell activation). In addition to being involved in effector cell responses, it has been found that such a molecule is involved in the response of the T cell to antigen.

In a preferred embodiment, the receptor on the surface of the T cell which mediates contact-dependent helper effector functions is gp39. In this embodiment, the antagonist is a molecule which inhibits the interaction of gp39 with its ligand on a cell which presents antigen to the T cell. A particularly preferred gp39 antagonist is an anti-gp39 antibody. Alternatively, the gp39 antagonist is a soluble form of a gp39 ligand, for example soluble CD40.

The method of the invention is based at least in part on the observation that administration of anti-gp39 antibodies to mice prevents induction of EAE and reverses the disease in animals having EAE. Thus, it has been found that an agent which inhibits the interaction of gp39 on a T cell with its ligands(s) on other cells is effective, both prophylactically and therapeutically, in treating a typical T cell mediated autoimmune disease. This result is surprising in view of previous studies which have attributed to gp39 a primary role in regulating B cell responses. The finding that anti-gp39 antibodies are effective in treating T cell mediated autoimmune disease forms the basis of the present invention. According to the invention, subjects having a T cell mediated autoimmune disease, such as multiple sclerosis, are treated by administration of agents that mimic the effect of anti-gp39 antibodies.

#### T Cell Mediated Autoimmune Diseases

The language "autoimmune disorder" is intended to include disorders in which the immune system of a subject reacts to autoantigens, such that significant tissue or cell destruction occurs in the subject. The term "autoantigen" is intended to include any antigen of a subject that is recognized by the immune system of the subject. The terms "autoantigen" and "self-antigen" are used interchangeably herein. The term "self" as used herein is intended to mean any component of a subject and includes molecules, cells, and organs. Autoantigens may be peptides, nucleic acids, or other biological substances. The language "T cell mediated autoimmune disorder" is intended to include autoimmune disorders in which the reaction to self primarily involves cell-mediated immune mechanisms, as opposed to humoral immune mechanisms. Thus, the methods of the invention pertain to treatments of autoimmune disorders in which tissue destruction is primarily mediated through activated T cells and immune cells other than B lymphocytes. However, even though the methods of the invention are intended for treatment of autoimmune disorders in which reaction to self is primarily mediated by cells other than B cells, the autoimmune disorders may be



characterized by the presence of autoantibodies. For example, EAE, a T cell mediated autoimmune disorder, which can be treated by a method of the invention, is frequently associated with the presence of autoantibodies to components of the central nervous system, such as myelin basic protein. Non limiting examples of T cell mediated autoimmune disorders that can be treated by the methods of the invention include multiple sclerosis, EAE, diabetes type I, oophoritis, and thyroiditis.

#### gp39 Antagonists

According to the methods of the invention, a gp39 antagonist is administered to a subject to interfere with the interaction of gp39 on T cells with a gp39 ligand on antigen presenting cells, such as B cells and thereby to prevent, alleviate or ameliorate the disorder. A gp39 antagonist is defined as a molecule which interferes with this interaction. As described more fully below, the gp39 antagonist can be an antibody directed against gp39 (e.g., a monoclonal antibody against gp39), a fragment or derivative of an antibody directed against gp39 (e.g., Fab or F(ab)'<sub>2</sub> fragments, chimeric antibodies or humanized antibodies), soluble forms of a gp39 ligand (e.g., soluble CD40), soluble forms of a fusion protein of a gp39 ligand (e.g., soluble CD40Ig), or pharmaceutical agents which disrupt or interfere with the gp39-CD40 interaction.

#### A. Antibodies

To prepare anti-gp39 antibodies, a mammal (e.g., a mouse, hamster, or rabbit) can be immunized with an immunogenic form of gp39 protein or protein fragment (e.g., peptide fragment) which elicits an antibody response in the mammal. A cell which expresses gp39 on its surface can also be used as the immunogen. Alternative immunogens include purified gp39 protein or protein fragments. gp39 can be purified from a gp39-expressing cell by standard purification techniques, e.g., gp39 cDNA (Armitage et al., *Nature*, 357:80-82 (1992); Lederman et al., *J. Exp. Med.*, 175:1091-1101 (1992); Hollenbaugh et al., *EMBO J.*, 11:4313-4319 (1992)) can be expressed in a host cell, e.g., bacteria or a mammalian cell line, and gp39 protein purified from the cell culture by standard techniques. gp39 peptides can be synthesized based upon the amino acid sequence of gp39 (disclosed in Armitage et al., *Nature*, 357:80-82 (1992); Lederman et al., *J. Exp. Med.*, 175:1091-1101 (1992); Hollenbaugh et al., *EMBO J.*, 11:4313-4319 (1992)) using known techniques (e.g. F-moc or T-boc chemical synthesis). Techniques for conferring immunogenicity on a protein include conjugation to carriers or other techniques well known in the art. For example, the protein can be administered in the presence of adjuvant. The progress of immunization can be monitored by detection of antibody titers in plasma or serum. Standard ELISA or other immunoassay can be used with the immunogen as antigen to assess the levels of antibodies.

Following immunization, antisera can be obtained and, if desired, polyclonal antibodies isolated from the sera. To produce monoclonal antibodies, antibody producing

cells (lymphocytes) can be harvested from an immunized animal and fused with myeloma cells by standard somatic cell fusion procedures thus immortalizing these cells and yielding hybridoma cells. Such techniques are well known in the art. For example, the hybridoma technique originally developed by Kohler and Milstein (*Nature* (1975) 256:495-497) as well as other techniques such as the human B-cell hybridoma technique (Kozbar et al., *Immunol. Today* (1983) 4:72), the EBV-hybridoma technique to produce human monoclonal antibodies (Cole et al. *Monoclonal Antibodies in Cancer Therapy* (1985) (Allen R. Bliss, Inc., pages 77-96), and screening of combinatorial antibody libraries (Huse et al., *Science* (1989) 246:1275). Hybridoma cells can be screened immunochemically for production of antibodies specifically reactive with the protein or peptide and monoclonal antibodies isolated.

The term antibody as used herein is intended to include fragments thereof which are specifically reactive with a gp39 protein or peptide thereof or gp39 fusion protein. Antibodies can be fragmented using conventional techniques and the fragments screened for utility in the same manner as described above for whole antibodies. For example, F(ab')<sub>2</sub> fragments can be generated by treating antibody with pepsin. The resulting F(ab')<sub>2</sub> fragment can be treated to reduce disulfide bridges to produce Fab' fragments. The antibody of the present invention is further intended to include bispecific and chimeric molecules having an anti-gp39 portion.

When antibodies produced in non-human subjects are used therapeutically in humans, they are recognized to varying degrees as foreign and an immune response may be generated in the patient. One approach for minimizing or eliminating this problem, which is preferable to general immunosuppression, is to produce chimeric antibody derivatives, i.e., antibody molecules that combine a non-human animal variable region and a human constant region. Chimeric antibody molecules can include, for example, the antigen binding domain from an antibody of a mouse, rat, or other species, with human constant regions. A variety of approaches for making chimeric antibodies have been described and can be used to make chimeric antibodies containing the immunoglobulin variable region which recognizes gp39. See, for example, Morrison et al., *Proc. Natl. Acad. Sci. U.S.A.* 81:6851 (1985); Takeda et al., *Nature* 314:452 (1985), Cabilly et al., U.S. Patent No. 4,816,567; Boss et al., U.S. Patent No. 4,816,397; Tanaguchi et al., European Patent Publication EP171496; European Patent Publication 0173494, United Kingdom Patent GB 2177096B. It is expected that such chimeric antibodies would be less immunogenic in a human subject than the corresponding non-chimeric antibody.

For human therapeutic purposes the monoclonal or chimeric antibodies specifically reactive with a gp39 protein or peptide can be further humanized by producing human variable region chimeras, in which parts of the variable regions, especially the conserved framework regions of the antigen-binding domain, are of human origin and only the hypervariable regions are of non-human origin. Such altered immunoglobulin molecules may be made by any of several techniques known in the art, (e.g., Teng et al., *Proc. Natl. Acad.*

*Sci. U.S.A.*, 80:7308-7312 (1983); Kozbor et al., *Immunology Today*, 4:7279 (1983); Olsson et al., *Meth. Enzymol.*, 92:3-16 (1982)), and are preferably made according to the teachings of PCT Publication WO92/06193 or EP 0239400. Humanized antibodies can be commercially produced by, for example, Scotgen Limited, 2 Holly Road, Twickenham, Middlesex, Great Britain.

Another method of generating specific antibodies, or antibody fragments, reactive against a gp39 protein or peptide is to screen expression libraries encoding immunoglobulin genes, or portions thereof, expressed in bacteria with a gp39 protein or peptide. For example, complete Fab fragments, VH regions and FV regions can be expressed in bacteria using phage expression libraries. See for example Ward et al., *Nature*, 341: 544-546: (1989); Huse et al., *Science*, 246: 1275-1281 (1989); and McCafferty et al., *Nature*, 348: 552-554 (1990). Screening such libraries with, for example, a gp39 peptide can identify immunoglobulin fragments reactive with gp39. Alternatively, the SCID-hu mouse (available from Genpharm) can be used to produce antibodies, or fragments thereof.

Methodologies for producing monoclonal antibodies directed against gp39, including human gp39 and mouse gp39, and suitable monoclonal antibodies for use in the methods of the invention, are described in PCT Patent Application No. WO 95/06666 entitled "Anti-gp39 Antibodies and Uses Therefor", the teachings of which are incorporated by reference. Particularly preferred anti-human gp39 antibodies of the invention are mAbs 24-31 and 89-76, produced respectively by hybridomas 24-31 and 89-76. The 89-76 and 24-31 hybridomas, producing the 89-76 and 24-31 antibodies, respectively, were deposited under the provisions of the Budapest Treaty with the American Type Culture Collection, Parklawn Drive, Rockville, Md., on September 2, 1994. The 89-76 hybridoma was assigned ATCC Accession Number HB11713 and the 24-31 hybridoma was assigned ATCC Accession Number HB11712.

Recombinant anti-gp39 antibodies, such as chimeric and humanized antibodies, can be produced by manipulating nucleic acid (e.g., DNA) encoding an anti-gp39 antibody according to standard recombinant DNA techniques. Accordingly, another aspect of this invention pertains to isolated nucleic acid molecules encoding immunoglobulin heavy or light chains, or portions thereof, reactive with gp39, particularly human gp39. The immunoglobulin-encoding nucleic acid can encode an immunoglobulin light or heavy chain variable region, with or without a linked heavy or light chain constant region (or portion thereof). Such nucleic acid can be isolated from a cell (e.g., hybridoma) producing an anti-human gp39 mAb by standard techniques. For example, nucleic acid encoding the 24-31 or 89-76 mAb can be isolated from the 24-31 or 89-76 hybridoma, respectively, by cDNA library screening, PCR amplification or other standard technique. Following isolation of, and possible further manipulation of, Moreover, nucleic acid encoding an anti-human gp39 mAb can be incorporated into an expression vector and introduced into a host cell to facilitate expression and production of recombinant forms of anti-human gp39 antibodies.

### B. Soluble Ligands for gp39

Other gp39 antagonists which can be used to induce T cell tolerance are soluble forms of a gp39 ligand. A monovalent soluble ligand of gp39, such as soluble CD40 can bind gp39, thereby inhibiting the interaction of gp39 with CD40 on B cells. The term "soluble" indicates that the ligand is not permanently associated with a cell membrane. A soluble gp39 ligand can be prepared by chemical synthesis, or, preferably by recombinant DNA techniques, for example by expressing only the extracellular domain (absent the transmembrane and cytoplasmic domains) of the ligand. A preferred soluble gp39 ligand is soluble CD40. Alternatively, a soluble gp39 ligand can be in the form of a fusion protein. Such a fusion protein comprises at least a portion of the gp39 ligand attached to a second molecule. For example, CD40 can be expressed as a fusion protein with immunoglobulin (i.e., a CD40Ig fusion protein). In one embodiment, a fusion protein is produced comprising amino acid residues of an extracellular domain portion of the CD40 molecule joined to amino acid residues of a sequence corresponding to the hinge, CH2 and CH3 regions of an immunoglobulin heavy chain, e.g., C $\gamma$ 1, to form a CD40Ig fusion protein (see e.g., Linsley et al. (1991) *J. Exp. Med.* 178:721-730; Capon et al. (1989) *Nature* 337, 525-531; and Capon U.S. 5,116,964). The fusion protein can be produced by chemical synthesis, or, preferably by recombinant DNA techniques based on the cDNA of CD40 (Stamenkovic et al., *EMBO J.*, 8:1403-1410 (1989)).

An antagonist of the invention is administered to subjects in a biologically compatible form suitable for pharmaceutical administration *in vivo*. By "biologically compatible form suitable for administration *in vivo*" is meant a form of the antagonist to be administered in which any toxic effects are outweighed by the therapeutic effects of the protein. The term subject is intended to include living organisms in which an immune response can be elicited, e.g., mammals. Examples of subjects include humans, dogs, cats, mice, rats, and transgenic species thereof. A gp39 antagonist can be administered in any pharmacological form, optionally in a pharmaceutically acceptable carrier. Administration of a therapeutically active amount of the antagonist is defined as an amount effective, at dosages and for periods of time necessary to achieve the desired result. For example, a therapeutically active amount of an antagonist of gp39 may vary according to factors such as the disease state, age, sex, and weight of the individual, and the ability of the antagonist to elicit a desired response in the individual. Dosage regimens may be adjusted to provide the optimum therapeutic response. For example, several divided doses may be administered daily or the dose may be proportionally reduced as indicated by the exigencies of the therapeutic situation.

The active compound (e.g., antagonist) may be administered in a convenient manner such as by injection (subcutaneous, intravenous, etc.), oral administration, inhalation, transdermal application, or rectal administration. Depending on the route of administration, the active compound may be coated in a material to protect the compound from the action of

enzymes, acids and other natural conditions which may inactivate the compound. A preferred route of administration is by intravenous injection.

To administer an antagonist of gp39 by other than parenteral administration, it may be necessary to coat the antagonist with, or co-administer the antagonist with, a material to prevent its inactivation. For example, an antagonist can be administered to an individual in an appropriate carrier or diluent, co-administered with enzyme inhibitors or in an appropriate carrier such as liposomes. Pharmaceutically acceptable diluents include saline and aqueous buffer solutions. Enzyme inhibitors include pancreatic trypsin inhibitor, diisopropyl-fluorophosphate (DEP) and trasylol. Liposomes include water-in-oil-in-water emulsions as well as conventional liposomes (Strejan et al., (1984) *J. Neuroimmunol* 7:27).

The active compound may also be administered parenterally or intraperitoneally. Dispersions can also be prepared in glycerol, liquid polyethylene glycols, and mixtures thereof and in oils. Under ordinary conditions of storage and use, these preparations may contain a preservative to prevent the growth of microorganisms.

Pharmaceutical compositions suitable for injectable use include sterile aqueous solutions (where water soluble) or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersion. In all cases, the composition must be sterile and must be fluid to the extent that easy syringability exists. It must be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms such as bacteria and fungi. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyethylene glycol, and the like), and suitable mixtures thereof. The proper fluidity can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. Prevention of the action of microorganisms can be achieved by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, asorbic acid, thimerosal, and the like. In many cases, it will be preferable to include isotonic agents, for example, sugars, polyalcohols such as manitol, sorbitol, sodium chloride in the composition. Prolonged absorption of the injectable compositions can be brought about by including in the composition an agent which delays absorption, for example, aluminum monostearate and gelatin.

Sterile injectable solutions can be prepared by incorporating active compound (e.g., an antagonist of gp39) in the required amount in an appropriate solvent with one or a combination of ingredients enumerated above, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the active compound into a sterile vehicle which contains a basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum drying and freeze-

drying which yields a powder of the active ingredient (e.g., antagonist) plus any additional desired ingredient from a previously sterile-filtered solution thereof.

When the active compound is suitably protected, as described above, the protein may be orally administered, for example, with an inert diluent or an assimilable edible carrier. As used herein "pharmaceutically acceptable carrier" includes any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like. The use of such media and agents for pharmaceutically active substances is well known in the art. Except insofar as any conventional media or agent is incompatible with the active compound, use thereof in the therapeutic compositions is contemplated.

Supplementary active compounds can also be incorporated into the compositions.

It is especially advantageous to formulate parenteral compositions in dosage unit form for ease of administration and uniformity of dosage. Dosage unit form as used herein refers to physically discrete units suited as unitary dosages for the mammalian subjects to be treated; each unit containing a predetermined quantity of active compound calculated to produce the desired therapeutic effect in association with the required pharmaceutical carrier. The specification for the dosage unit forms of the invention are dictated by and directly dependent on (a) the unique characteristics of the active compound and the particular therapeutic effect to be achieved, and (b) the limitations inherent in the art of compounding such an active compound for the treatment of sensitivity in individuals.

This invention is further illustrated by the following examples which should not be construed as limiting. The contents of all references, patents and published patent applications cited throughout this application are hereby incorporated by reference.

#### **EXAMPLE 1: EAE prevention by anti-gp39 antibody administration**

This example demonstrates that administration of anti-gp39 antibodies to mice prevents induction of experimental allergic encephalomyelitis (EAE), an animal model for multiple sclerosis.

EAE is a well characterized model of a T cell mediated autoimmune disease and is an instructive model for the human autoimmune disease multiple sclerosis. EAE can be induced in susceptible animals, such as mice, by immunizing the animals with myelin basic protein (MBP), proteolipid protein (PLP), myelin oligodendrocyte protein (MOG), or synthetic peptides based on the sequences of these myelin associated proteins together with an adjuvant containing pertussis bacteria. One to two weeks after immunization, the animals develop encephalomyelitis, characterized by perivascular infiltrates containing lymphocytes and macrophages and the development of demyelination in the brain and the spinal cord. The animals show acute, chronic or chronic relapsing paralysis. In this example, the effect of administration of anti-gp39 antibodies on development of EAE in susceptible mice was analyzed.

EAE was induced in susceptible mice by subcutaneous injections (day 0) of an emulsion containing 70 µg or 300 µg PLP-peptide in 50 µl PBS and 25µg *Mycobacteria tuberculosis* (H37RA, Difco) in 50 µl of complete Freuds adjuvant at two sites in the abdominal flanks of the mice. 200 µl *Bordetella pertussis* suspension ( $10 \cdot 10^{10}$  in 1 ml PBS) was given intravenously at the same time as the peptide and two days later. The PLP-peptide injected in the mice has an amino acid sequence corresponding to amino acid residues 139 to 151 of rat PLP (Dautigny et al., *FEBS Lett.* 188:33, 1985). PLP-peptide was synthesized with f-moc protected aminoacids according to the solid phase synthesis method (Merrifield, *J. Am. Chem. Soc.* 85:2149, 1963). Immunization with this peptide results in the development of acute EAE, which is clinically and pathologically identical to that induced by sensitization with whole central nervous system (CNS) myelin or with MBP (Tuohy et al., *J. Immunol.* 142:1523, 1989; Sobel et al., *J. Neuropathol. Exp. Neurol.* 49:468, 1990).

To determine the effect of anti-gp39 on the course of the disease, mice were injected on day 0 with PLP-peptide, as described above, and further injected intraperitoneally with 125 µg hamster anti-gp39 Mabs (Noelle et al., *Proc. Natl. Acad. Sci. USA* 89:6550, 1992) in 200 µl PBS or with 125 µg normal hamster antibodies (Serva Feinbiochemica) in 200 µl PBS (control animals) on days 0, 2, and 4. The severity of EAE clinical signs was evaluated each day and graded according to average disability scale (DAS) scores: grade 0 = no clinical signs, grade 1 = tail weakness, grade 2 = mild paraparesis and ataxia of the hind legs, grade 3 = severe paraparesis or ataxia of the hind legs, grade 4 = moribund, grade 5 = dead due to EAE.

Figure 1 represents the course of the disease in mice injected with 70 µg of PLP-peptide (Panel A) or with 300 µg of PLP-peptide (Panel B) and treated with anti-gp39 or control antibody. DAS scores, which reflect the severity of the disease, of control mice and anti-gp39 treated mice are depicted in grey and black bars respectively.

The results indicate that animals having received the control antibody developed EAE, whereas animals having received anti-gp39 antibody were protected from induction of the disease. For animals having received control antibody, the first clinical signs of EAE became apparent on day eleven. In these animals, the highest DAS score was 2.33, which was observed on days 15-22 in animals injected with 75 µg of PLP-peptide (Fig.1, Panel A, grey bars) and 3.6, observed on days 16-23 in animals injected with 300 µg PLP-peptide (Fig. 1, Panel B, grey bars). In contrast, animals which received the anti-gp39 monoclonal antibodies showed no clinical signs after induction of EAE with 75 µg of PLP-peptide (Fig 1, Panel A, black bars) and only minor clinical signs, which completely disappeared at day 31, after induction of the disease with 300 µg of PLP-peptide (Fig 1, Panel B, black bars).

Thus, administration of anti-gp39 antibodies to mice completely inhibited induction of EAE in these mice.

Induction of EAE by adoptive transfer of myelin reactive T-cells isolated from animals immunized with myelin components or obtained after *in vitro* activation with myelin

components indicates that especially activated T-cells are responsible for the development of clinical characteristics after the inductive phase (Pettinelli and McFarlin, *J. Immunol.* 127:1420, 1979; Mokhtarian et al., *Nature* 309:356, 1984; Veen et al., *J. Neuroimmunol.* 21:183, 1989). However, the experiments described herein show that administration of anti-gp39 monoclonal antibodies prevents the development of EAE. In control groups, significant anti-PLP-peptide antibody responses were observed on day 14 (absorbance 1.92) and 21 (absorbance 2.15) in animals in which EAE was induced with low and high PLP-peptide dose, respectively. In contrast, significant anti-PLP-peptide antibody responses in the gp39 treated animals were observed first on day 14, and reached plateau levels on day 31 (absorbance 0.928) and 40 (absorbance 1.54) in animals which were injected with low and high PLP-peptide dose, respectively. The generation of significant anti-PLP-peptide antibody responses in anti-gp39 monoclonal antibodies treated mice, was postponed until day 14, which indicates that the anti-gp39 monoclonal antibodies had some effect on antibody production.

Thus, this example demonstrates that anti-gp39 prevent development of EAE and indicate that anti-gp39 antibodies can be used for treating T cell mediated autoimmune diseases, such as multiple sclerosis.

#### **EXAMPLE 2: Reversal of EAE by anti-gp39 antibody administration**

Example 1 showed the inhibitory effect of anti-gp39 antibody on induction of EAE. Thus, it was demonstrated that immunization of the mice at the time of induction of the disease prevented development of the disease. This example shows that administration of the antibody after induction of the disease leads to regression of the disease.

In this example, EAE was induced in female SJL/j mice (10-12 weeks old) by injection of an emulsion containing 150 µg PLP-peptide prepared as described above. For determining the effect of the anti-gp39 antibodies when administered to the mice after induction of the disease, mice were injected intraperitoneally with 125 µg anti-gp39 monoclonal antibodies (Noelle et al., *Proc. Natl. Acad. Sci. USA* 89:6550, 1992) in 200 µl of PBS (anti-gp39 treated mice) or with 200 µl PBS alone (control mice) on days 0, 2 and 4, on days 4, 6 and 8, or on days 7, 9 and 11. The results are presented as percentage suppression, and are a comparison of the total of daily DAS scores (from day 12 to 28) in anti-gp39 treated animals and in control animals.

The results, which are presented in Figure 2, indicate that administration of the first dose of anti-gp39 antibodies as late as 7 days after injection into the mice of PLP-peptide results in more than 60% suppression of the disease. Thus, anti-gp39 antibodies are capable of reversing or suppressing EAE.

The results further indicate that even though administration of a first dose of antibody only 7 days after induction of the disease in mice results in significant suppression of



development of the disease, anti-gp39 treatment is somewhat more efficient when the first dose of antibody is administered sooner after induction of the disease.

Thus, administration of anti-gp39 antibodies to mice protects these mice from developing EAE upon induction of the disease and suppresses the disease in mice having EAE.

**EXAMPLE 3:      Suppression of EAE after spleen cell transfer of gp39 treated mice**

Regulatory suppressor T-cells have been detected in Lewis rat, which have been recovered from EAE (Pessoa et al., *J. Neuroimmunol.* 7:131, 1984) and after oral administration of myelin components (Lider et al., *J. Immunol.* 142:748, 1989; Hafler et al., *Ann. NY Acad. Sci.* 636:251, 1991). It was postulated by Karpus and Swanborg (*J. Immunol.* 143:3492, 1989) that CD4<sup>+</sup> suppressor T-cells isolated from rats which have recovered from EAE, can down regulate EAE T-effector cells by differential inhibition of lymphokine production. In contrast, suppression of EAE in Lewis rats by oral administration of MBP is mediated by CD8<sup>+</sup> T-cells (Miller et al., *J. Exp. Med.* 174:791, 1991). To determine whether T cells of mice having been protected from EAE by administration of anti-gp39 antibody, are capable of protecting naive animals from EAE, the following example was undertaken.

In this example, a first set of mice were injected with 150 µg of PLP-peptide and a second set of mice were injected with 150 µg of PLP-peptide and anti-gp39 antibody according to the protocol described in Example 1. Four months later the mice were sacrificed by CO<sub>2</sub> euthanasia and the spleens were removed. Erythrocytes were removed by standard ammoniumchloride treatment (Mishell and Shiigi, *Selected Methods in Cellular Immunology*, W.H. Freeman and Company, 1980). Cells of individual spleens (500 µl) were injected i.v. in naive 5 Gy irradiated recipient female SLJ/j mice (10-12 weeks old). Two days after cell transfer, mice were challenged by intraperitoneal injection with 150 µg PLP-peptide following the procedure described in Example 1 and DAS scores determined.

Figure 3 represents the DAS scores of the animals. The results demonstrate that mice that have been transplanted with spleen cells from animals initially injected with PLP-peptide and anti-gp39 antibodies are protected from development of the disease, whereas mice transplanted with spleen cells from animals initially injected with PLP-peptide only develop EAE. Moreover, considering that the estimated half-life of antibodies is 12 days, it may be expected that no antibodies were present in the spleen cells transplanted in the mice. Therefore, the protective effect conferred by donor spleen cells from animals having received PLP-peptide and anti-gp39 antibodies cannot be explained by the presence of anti-gp39 antibodies. DAS scores of these mice indicates that suppression of EAE in the recipient mice is most likely due to the presence of a T-suppressor cell population in the transferred spleen cell suspension and that this T-suppressor cell population overrules the T-effector cell population efficiently.

**EXAMPLE 4: Detection of gp39 positive Th-cells**

This example demonstrates the presence of gp39 positive cells in the central nervous system of human subjects having multiple sclerosis.

Human autopsy central nervous system (CNS) tissues were obtained from the Netherlands brain bank, Amsterdam, the Netherlands. Gp39 positive cells were detected with an CD40-Ig fusion protein according to methods known in the art. CNS tissue sections of an MS patient and an Alzheimer patient were stained with CD40-Ig. In this example, only CNS tissues of MS-patients in which anti-MBP antibody forming cells were detected previously were used. The results of the staining show the presence of gp39 positive cells in a 8  $\mu$ m coronal cerebrum section of an MS patient, but no gp39 positive cells were detected in coronal cerebrum sections of Alzheimer patients. Thus, gp39 positive cells were detected only in CNS tissue sections of MS patients. The presence of gp39 positive cells in MS-patient CNS tissue together with the detection of anti-MBP antibody forming cells in CNS tissues of MS-patients only and not in control CNS tissues indicates that such cells play an role in the pathological affected CNS tissues of MS patients.

In this study we have shown that suppression of B-cell activation by the administration of anti-gp39 Mabs, can result in the complete prevention of EAE development, dependent on the dose of antigen by which EAE was induced and dependent on the time period between EAE induction and administration of anti-gp39 Mabs. Although the exact mechanisms responsible for EAE induction and development are not clear, these data indicate that anti-gp39 antibody can be used for treatment of autoimmune diseases.

**Equivalents**

Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents of the specific embodiments of the invention described herein. Such equivalents are intended to be encompassed by the following claims.

**Claims**

1. A method for treating a T cell mediated autoimmune disorder comprising administering to the subject a therapeutically or prophylactically effective amount of an antagonist of a receptor on a surface of a T cell which mediates contact dependent helper effector functions.
2. A method of claim 1, wherein the T cell mediated autoimmune disorder is selected from the group consisting of multiple sclerosis, EAE, diabetes type I, oophoritis and thyroiditis.
3. A method of claim 1, wherein the T cell mediated autoimmune disorder is multiple sclerosis.
4. The method of claim 1, wherein the receptor on the surface of the T cell which mediates contact-dependent helper effector function is gp39.
5. The method of claim 4, wherein the antagonist is an anti-gp39 antibody.
6. The method of claim 5, wherein the anti-gp39 antibody is a monoclonal antibody.
7. The method of claim 5, wherein the anti-gp39 antibody is an anti-human gp39 antibody.
8. The method of claim 6, wherein the monoclonal antibody is produced by 89-76 hybridoma, ATCC Accession Number HB11713 or 24-31 hybridoma, ATCC Accession Number HB11712 or an antibody having the gp39 binding characteristics thereof.
9. The method of claim 6, wherein the monoclonal antibody is a chimeric monoclonal antibody.
10. The method of claim 6, wherein the monoclonal antibody is a humanized monoclonal antibody.
11. A method of treating multiple sclerosis comprising administering to a subject a therapeutically effective amount of an anti-human gp39 antibody.

**Abstract**

Method for the treatment of multiple sclerosis and other T cell mediated autoimmune disorders is described. The method involves administering to a subject a therapeutically effective amount of an antagonist of a receptor on a surface of a T cell which mediates contact dependent helper effector functions, for example, an anti-gp39 antibody.

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60  
61  
62  
63  
64  
65  
66  
67  
68  
69  
70  
71  
72  
73  
74  
75  
76  
77  
78  
79  
80  
81  
82  
83  
84  
85  
86  
87  
88  
89  
90  
91  
92  
93  
94  
95  
96  
97  
98  
99  
100  
101  
102  
103  
104  
105  
106  
107  
108  
109  
110  
111  
112  
113  
114  
115  
116  
117  
118  
119  
120  
121  
122  
123  
124  
125  
126  
127  
128  
129  
130  
131  
132  
133  
134  
135  
136  
137  
138  
139  
140  
141  
142  
143  
144  
145  
146  
147  
148  
149  
150  
151  
152  
153  
154  
155  
156  
157  
158  
159  
160  
161  
162  
163  
164  
165  
166  
167  
168  
169  
170  
171  
172  
173  
174  
175  
176  
177  
178  
179  
180  
181  
182  
183  
184  
185  
186  
187  
188  
189  
190  
191  
192  
193  
194  
195  
196  
197  
198  
199  
200  
201  
202  
203  
204  
205  
206  
207  
208  
209  
210  
211  
212  
213  
214  
215  
216  
217  
218  
219  
220  
221  
222  
223  
224  
225  
226  
227  
228  
229  
230  
231  
232  
233  
234  
235  
236  
237  
238  
239  
240  
241  
242  
243  
244  
245  
246  
247  
248  
249  
250  
251  
252  
253  
254  
255  
256  
257  
258  
259  
260  
261  
262  
263  
264  
265  
266  
267  
268  
269  
270  
271  
272  
273  
274  
275  
276  
277  
278  
279  
280  
281  
282  
283  
284  
285  
286  
287  
288  
289  
290  
291  
292  
293  
294  
295  
296  
297  
298  
299  
300  
301  
302  
303  
304  
305  
306  
307  
308  
309  
310  
311  
312  
313  
314  
315  
316  
317  
318  
319  
320  
321  
322  
323  
324  
325  
326  
327  
328  
329  
330  
331  
332  
333  
334  
335  
336  
337  
338  
339  
340  
341  
342  
343  
344  
345  
346  
347  
348  
349  
350  
351  
352  
353  
354  
355  
356  
357  
358  
359  
360  
361  
362  
363  
364  
365  
366  
367  
368  
369  
370  
371  
372  
373  
374  
375  
376  
377  
378  
379  
380  
381  
382  
383  
384  
385  
386  
387  
388  
389  
390  
391  
392  
393  
394  
395  
396  
397  
398  
399  
400  
401  
402  
403  
404  
405  
406  
407  
408  
409  
410  
411  
412  
413  
414  
415  
416  
417  
418  
419  
420  
421  
422  
423  
424  
425  
426  
427  
428  
429  
430  
431  
432  
433  
434  
435  
436  
437  
438  
439  
440  
441  
442  
443  
444  
445  
446  
447  
448  
449  
450  
451  
452  
453  
454  
455  
456  
457  
458  
459  
460  
461  
462  
463  
464  
465  
466  
467  
468  
469  
470  
471  
472  
473  
474  
475  
476  
477  
478  
479  
480  
481  
482  
483  
484  
485  
486  
487  
488  
489  
490  
491  
492  
493  
494  
495  
496  
497  
498  
499  
500  
501  
502  
503  
504  
505  
506  
507  
508  
509  
510  
511  
512  
513  
514  
515  
516  
517  
518  
519  
520  
521  
522  
523  
524  
525  
526  
527  
528  
529  
530  
531  
532  
533  
534  
535  
536  
537  
538  
539  
540  
541  
542  
543  
544  
545  
546  
547  
548  
549  
550  
551  
552  
553  
554  
555  
556  
557  
558  
559  
560  
561  
562  
563  
564  
565  
566  
567  
568  
569  
570  
571  
572  
573  
574  
575  
576  
577  
578  
579  
580  
581  
582  
583  
584  
585  
586  
587  
588  
589  
590  
591  
592  
593  
594  
595  
596  
597  
598  
599  
600  
601  
602  
603  
604  
605  
606  
607  
608  
609  
610  
611  
612  
613  
614  
615  
616  
617  
618  
619  
620  
621  
622  
623  
624  
625  
626  
627  
628  
629  
630  
631  
632  
633  
634  
635  
636  
637  
638  
639  
640  
641  
642  
643  
644  
645  
646  
647  
648  
649  
650  
651  
652  
653  
654  
655  
656  
657  
658  
659  
660  
661  
662  
663  
664  
665  
666  
667  
668  
669  
670  
671  
672  
673  
674  
675  
676  
677  
678  
679  
680  
681  
682  
683  
684  
685  
686  
687  
688  
689  
690  
691  
692  
693  
694  
695  
696  
697  
698  
699  
700  
701  
702  
703  
704  
705  
706  
707  
708  
709  
710  
711  
712  
713  
714  
715  
716  
717  
718  
719  
720  
721  
722  
723  
724  
725  
726  
727  
728  
729  
730  
731  
732  
733  
734  
735  
736  
737  
738  
739  
740  
741  
742  
743  
744  
745  
746  
747  
748  
749  
750  
751  
752  
753  
754  
755  
756  
757  
758  
759  
760  
761  
762  
763  
764  
765  
766  
767  
768  
769  
770  
771  
772  
773  
774  
775  
776  
777  
778  
779  
780  
781  
782  
783  
784  
785  
786  
787  
788  
789  
790  
791  
792  
793  
794  
795  
796  
797  
798  
799  
800  
801  
802  
803  
804  
805  
806  
807  
808  
809  
810  
811  
812  
813  
814  
815  
816  
817  
818  
819  
820  
821  
822  
823  
824  
825  
826  
827  
828  
829  
830  
831  
832  
833  
834  
835  
836  
837  
838  
839  
840  
841  
842  
843  
844  
845  
846  
847  
848  
849  
850  
851  
852  
853  
854  
855  
856  
857  
858  
859  
860  
861  
862  
863  
864  
865  
866  
867  
868  
869  
870  
871  
872  
873  
874  
875  
876  
877  
878  
879  
880  
881  
882  
883  
884  
885  
886  
887  
888  
889  
890  
891  
892  
893  
894  
895  
896  
897  
898  
899  
900  
901  
902  
903  
904  
905  
906  
907  
908  
909  
910  
911  
912  
913  
914  
915  
916  
917  
918  
919  
920  
921  
922  
923  
924  
925  
926  
927  
928  
929  
930  
931  
932  
933  
934  
935  
936  
937  
938  
939  
940  
941  
942  
943  
944  
945  
946  
947  
948  
949  
950  
951  
952  
953  
954  
955  
956  
957  
958  
959  
960  
961  
962  
963  
964  
965  
966  
967  
968  
969  
970  
971  
972  
973  
974  
975  
976  
977  
978  
979  
980  
981  
982  
983  
984  
985  
986  
987  
988  
989  
990  
991  
992  
993  
994  
995  
996  
997  
998  
999  
1000

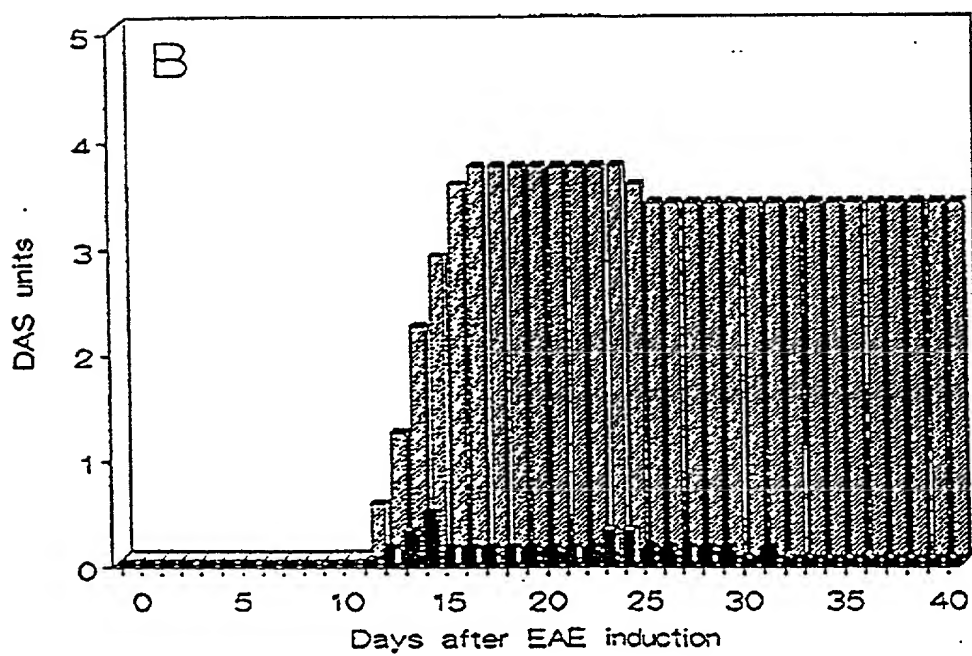
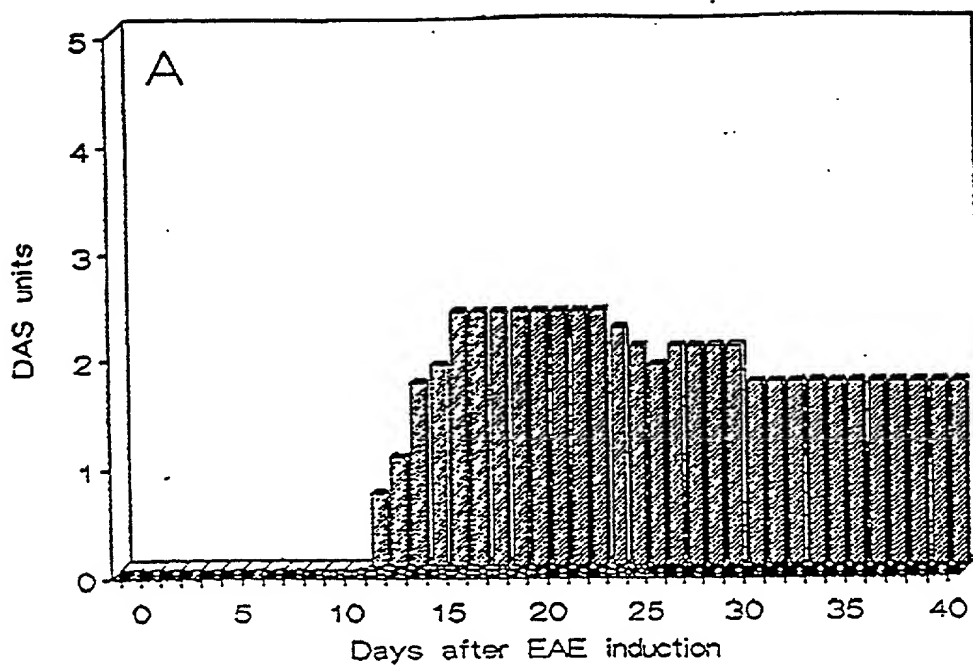


FIGURE 1

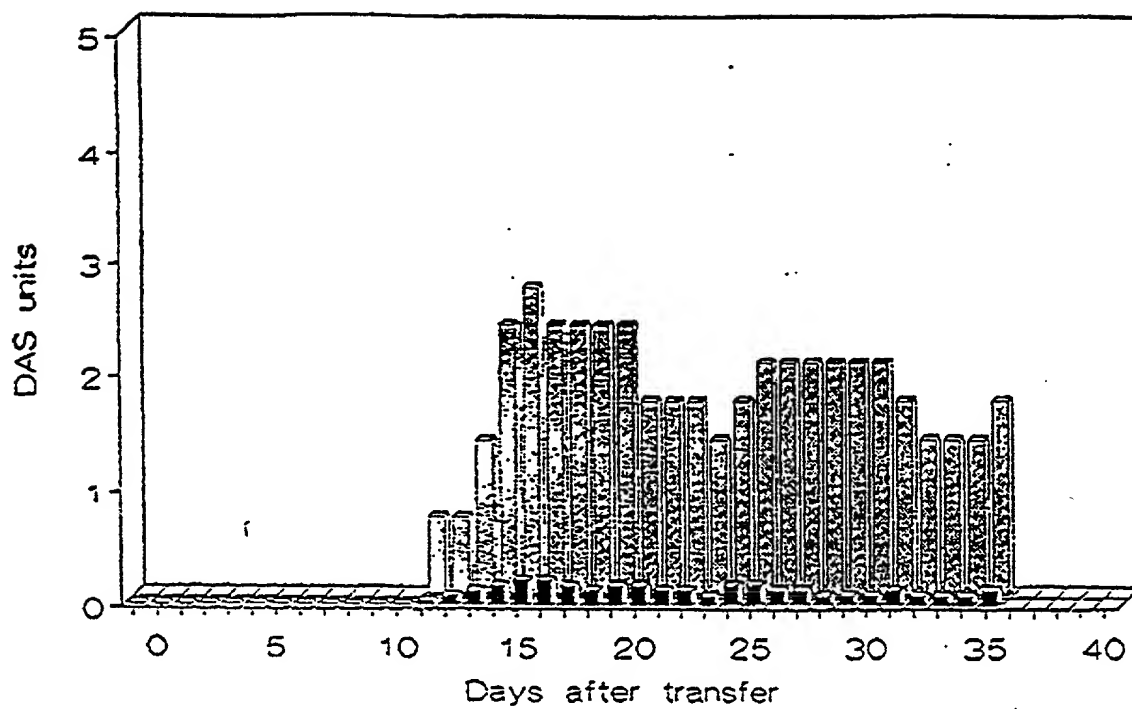


FIG. 3

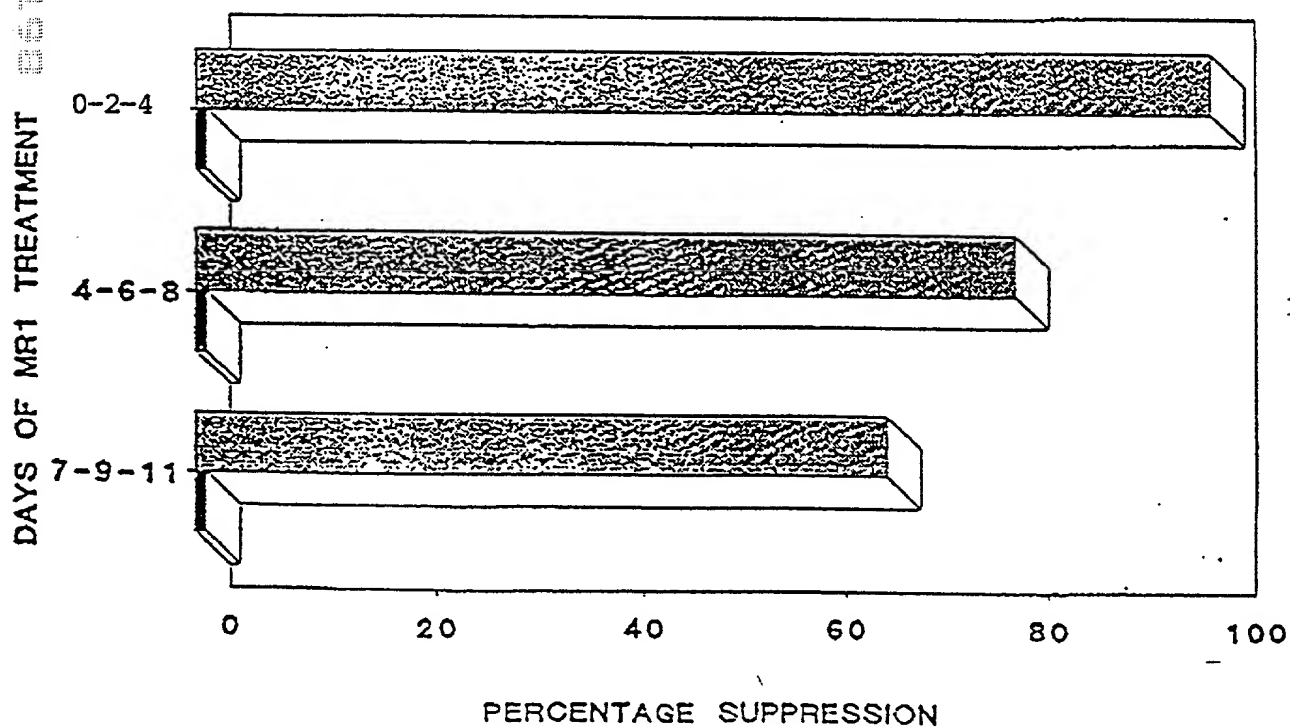


FIG. 2

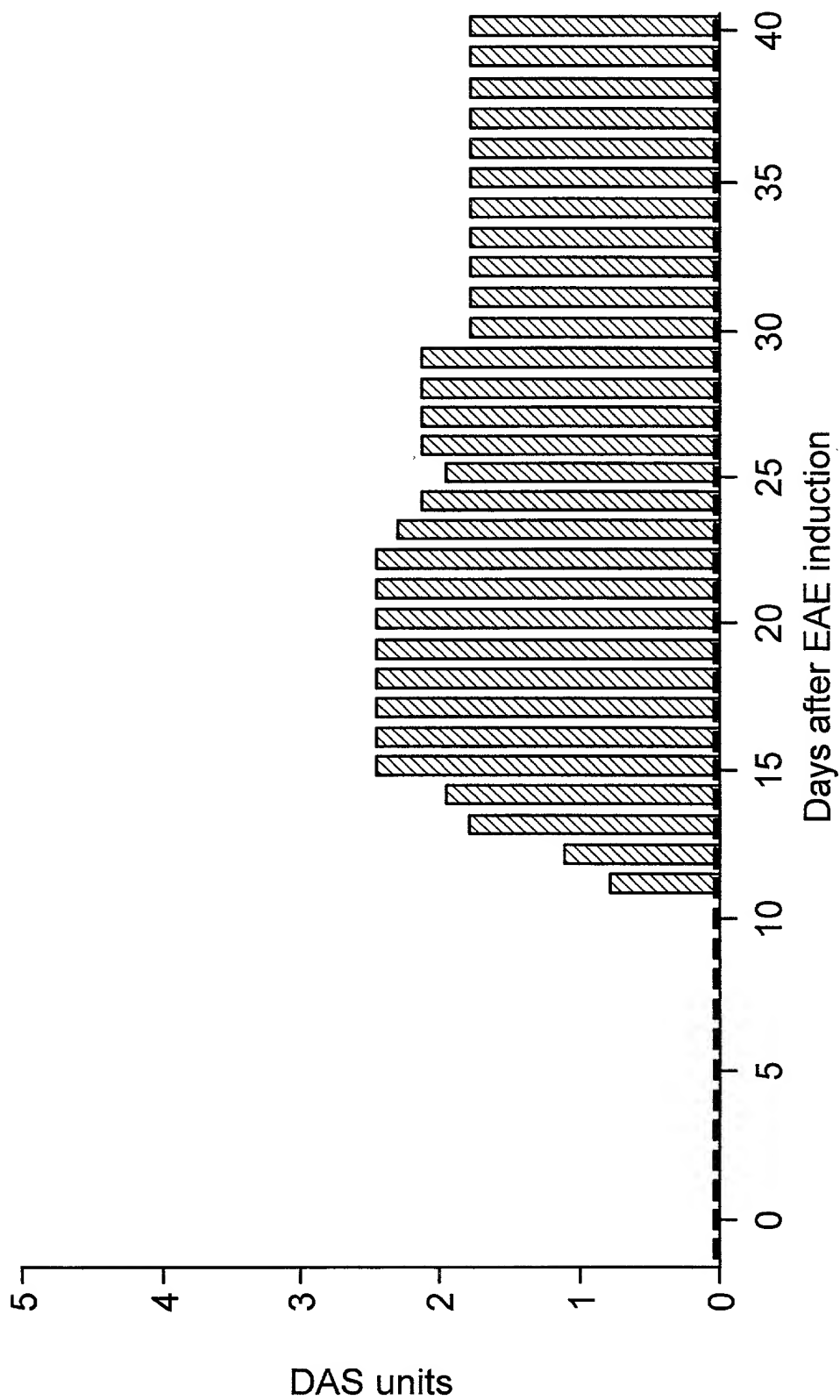


FIG. 1A

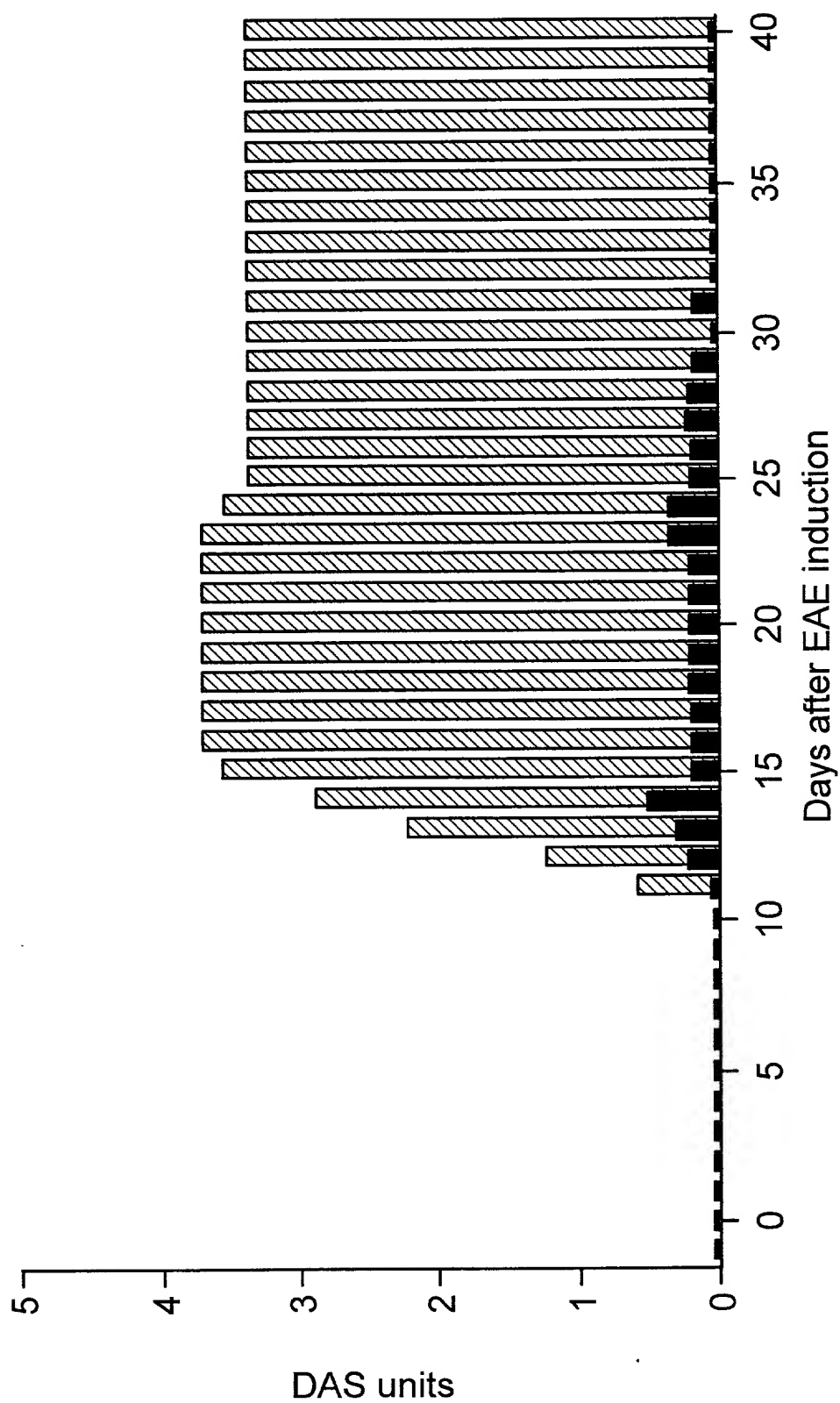
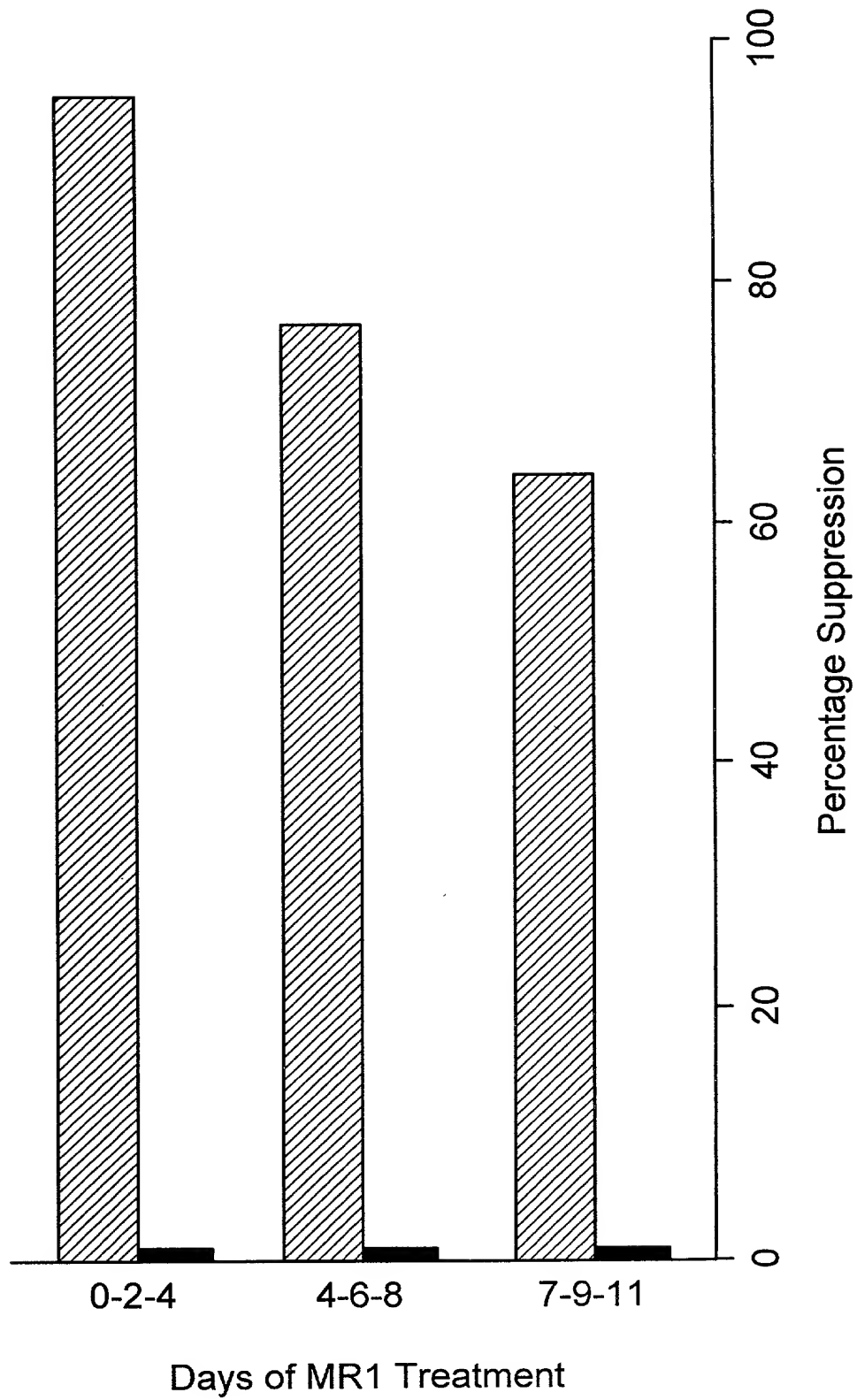


FIG. 1B



FIG. 2



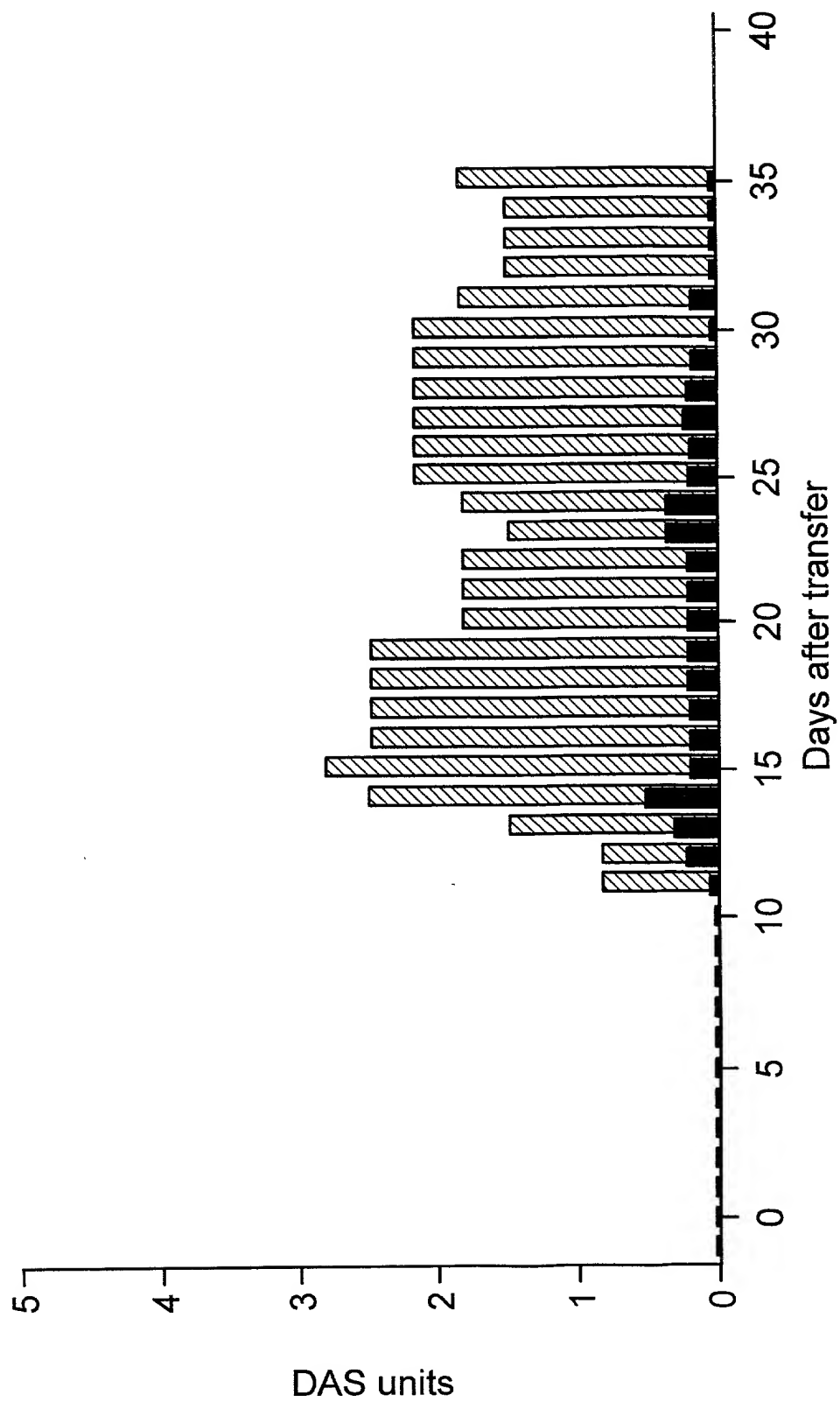


FIG. 3